

# Whole-transcriptome sequencing identifies a distinct subtype of acute lymphoblastic leukemia with predominant genomic abnormalities of *EP300* and *CREBBP*

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Chromosomal translocations are a genomic hallmark of many hematologic malignancies. Often as initiating events, these structural abnormalities result in fusion proteins involving transcription factors important for hematopoietic differentiation and/or signaling molecules regulating cell proliferation and cell cycle. In contrast, epigenetic regulator genes are more frequently targeted by somatic sequence mutations, possibly as secondary events to further potentiate leukemogenesis. Through comprehensive whole-transcriptome sequencing of 231 children with acute lymphoblastic leukemia (ALL), we identified 58 putative functional and predominant fusion genes in 54.1% of patients ( $n = 125$ ), 31 of which have not been reported previously. In particular, we described a distinct ALL subtype with a characteristic gene expression signature predominantly driven by chromosomal rearrangements of the *ZNF384* gene with histone acetyltransferases *EP300* and *CREBBP*. *ZNF384*-rearranged ALL showed significant up-regulation of *CLCF1* and *BTLA* expression, and *ZNF384* fusion proteins consistently showed higher activity to promote transcription of these target genes relative to wild-type *ZNF384* in vitro. Ectopic expression of *EP300-ZNF384* and *CREBBP-ZNF384* fusion altered differentiation of mouse hematopoietic stem and progenitor cells and also potentiated oncogenic transformation in vitro. *EP300*- and *CREBBP-ZNF384* fusions resulted in loss of histone lysine acetyltransferase activity in a dominant-negative fashion, with concomitant global reduction of histone acetylation and increased sensitivity of leukemia cells to histone deacetylase inhibitors. In conclusion, our results indicate that gene fusion is a common class of genomic abnormalities in childhood ALL and that recurrent translocations involving *EP300* and *CREBBP* may cause epigenetic deregulation with potential for therapeutic targeting.

[Supplemental material is available for this article.]

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Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and a leading cause of disease-related death in childhood (Inaba et al. 2013; Pui et al. 2015). While it is a prototype of cancer that can be cured by chemotherapy alone, current ALL therapy relies primarily on conventional cytotoxic agents with significant acute and long-term side effects (Robison 2011). Therefore, a better understanding of genomic landscape of ALL is critical for developing molecularly targeted therapy and implementing genomics-based precision medicine in this cancer. One of genomic hallmarks of ALL is chromosomal translocation that gives rise to tumor-specific fusion proteins with novel and often oncogenic functions (Wiemels 2008; Hunger and Mullighan 2015). For example, reciprocal translocation t(12;21) results in chimeric transcripts of two critical hematopoiesis transcription factors *ETV6* and *RUNX1*, and this fusion protein specifically promotes B-cell precursor expansion, enhances self-renewal capacity, and impairs B-lymphocyte differentiation (Hong et al. 2008; Schindler et al. 2009; Tsuzuki and Seto 2013). As initiating events, some of these large structural abnormalities arise early in life in children with ALL (Greaves and Wiemels 2003; Bateman et al. 2015) and frequently involve genes related to hematopoiesis and/or cell proliferation/cycling. Recent advances in genomic sequencing enabled the discovery of a variety of novel gene rearrangements in ALL, many of which are confined to these two pathways (Atak et al. 2013; Roberts and Mullighan 2015; Iacobucci et al. 2016; Yasuda et al. 2016).

Epigenetic modification is highly regulated during hematopoiesis and is important for proper maintenance of hierarchical differentiation (Ji et al. 2010; Abraham et al. 2013; Stergachis et al. 2013; Lara-Astiaso et al. 2014). However, chromosomal rearrangements in epigenetic regulator genes are relatively rare in hematologic malignancies. Instead, most somatic alterations in these genes are sequence mutations, possibly as a subsequent cooperating event. For example, *CREBBP* is recurrently mutated in ALL (Mullighan et al. 2011) and B-cell lymphoma (Pasqualucci et al. 2011), resulting in the loss of histone acetyltransferase (HAT) activity. While the exact biological consequences of these events remain unknown in the context of disease pathogenesis, these data point to the importance of the *EP300/CREBBP* signaling axis in normal and malignant hematopoiesis (Chalmers et al. 1990; Blobel 2000; Kimbrel et al. 2009). Mutations in histone genes themselves (e.g., histone gene cluster on 6p22) (Mullighan et al. 2007), histone modifying enzymes (e.g., *SETD2*, *ASXL2*, *KMT2D*, *KDM6A*) (Huether et al. 2014; Mar et al. 2014; Zhu et al. 2014), and chromatin remodeling genes (e.g., *ASXL1*, *EZH2*) (Nikoloski et al. 2010; Balasubramani et al. 2015) have also been reported, albeit with low frequencies. More recently, *EP300* fusion with the transcription factor *ZNF384* has been reported in ALL (Gocho et al. 2015; Ping et al. 2015; Yasuda et al. 2016), and *ZNF384* is also implicated in chromosomal translocation with a number of partner genes (Martini et al. 2002; La Starza et al. 2005; Zhong et al. 2008; Nyquist et al. 2011; Yasuda et al. 2016), although the biological functions of these fusions are largely unknown.

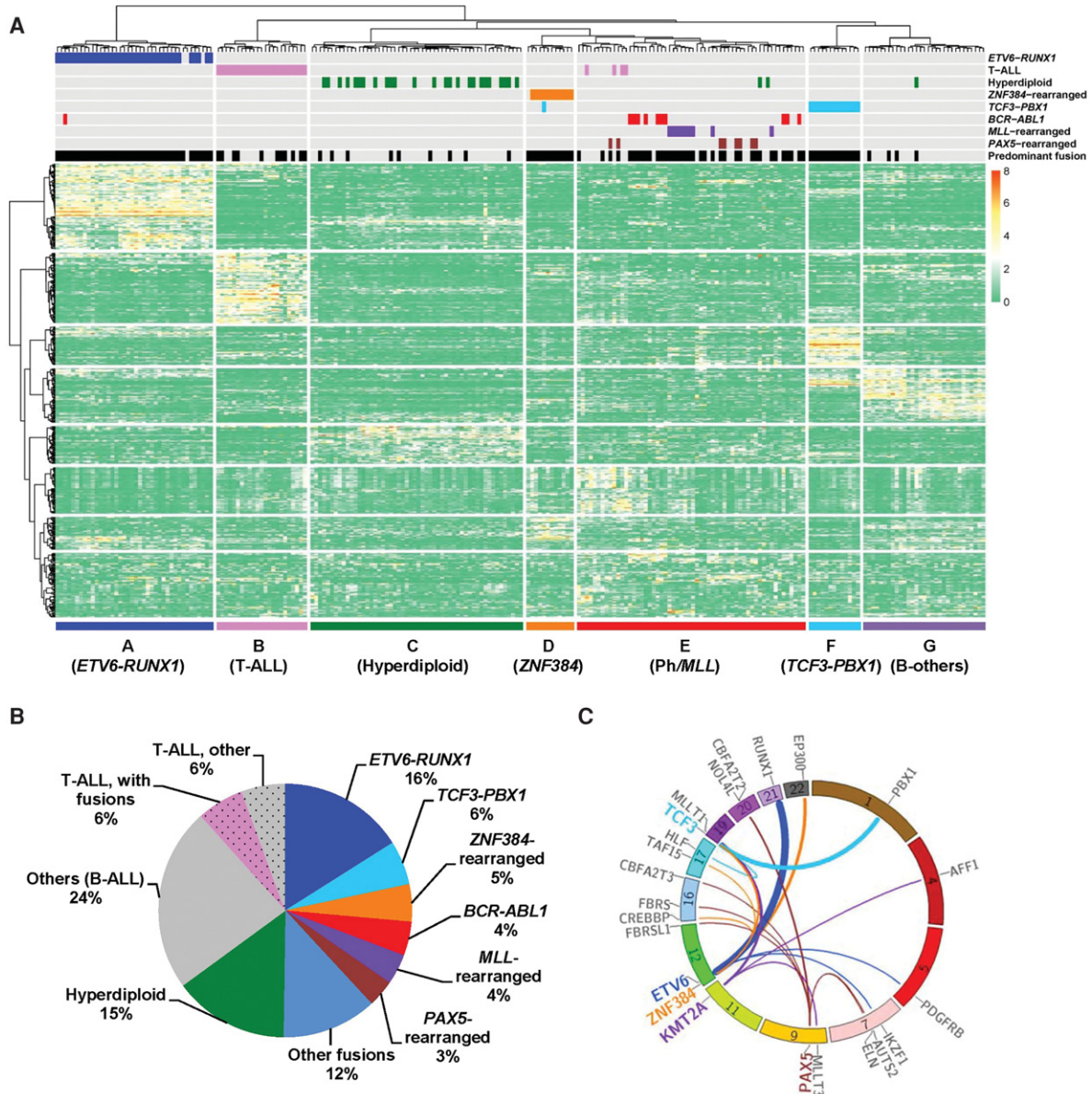
In the current study, we systematically described gene fusion events in childhood ALL, by whole-transcriptome sequencing, and identified a distinct ALL subtype characterized by *ZNF384* rearrangements and potential sensitivity to histone deacetylase inhibition.

## Results

We performed whole-transcriptome sequencing of 231 children with newly diagnosed ALL who were consecutively enrolled in

the Ma-Spore frontline ALL trials (Supplemental Table S1). Primarily of Asian descent (e.g., Chinese, Filipino, Indian, Malay), patients had a median age of 4.6 yr at diagnosis. Unsupervised hierarchical clustering and principal component analysis of global gene expression profile identified seven major ALL subgroups (Fig. 1A; Supplemental Fig. S1). Consistent with previous reports, cases with T-cell immunophenotype, *ETV6-RUNX1*, *TCF3-PBX1*, and hyperdiploid karyotype were readily identified by transcription signatures (Fig. 1A). Interestingly, ALL blasts from 12 patients did not carry these known cytogenetic abnormalities and formed a distinctive cluster. Further analyses revealed that 11 cases (91.6%) within this subgroup had gene fusion events involving the transcription factor *ZNF384* (Fig. 1A, B), all of which were confirmed by reverse transcription polymerase chain reaction (RT-PCR) amplification and Sanger sequencing (Supplemental Fig. S2). Overall, we have identified 130 fusion genes in 144 patients, 100 of which to our knowledge have not been reported previously (Supplemental Table S2). In 125 patients, a chimeric gene either was the only fusion event detected or was the clearly predominant fusion (58 unique fusions in total) (Supplemental Fig. S3). Thirty-seven predominant fusion genes (24 known and 13 novel) directly fused coding sequences of two genes with a predicted in-frame chimeric protein (Supplemental Fig. S3D). In contrast, 21 predominant fusion transcripts (three known and 18 novel) involved noncoding sequences from one or both partner genes, leading to aberrant expression of important signaling genes in some instances (e.g., *P2RY8-CRLF2*). Notably, *ETV6*, *PAX5*, *ZNF384*, *KMT2A*, and *TCF3* were repeatedly targeted by chromosomal translocations with diverse partner genes (Fig. 1C), highlighting their importance in ALL leukemogenesis.

In nine of 11 ALL cases with *ZNF384* rearrangements, the breakpoints in this gene were invariably between exon 2 and exon 3, resulting in deletion of only the 5' UTR and then in-frame fusion of the entire coding sequence with the other genes (Fig. 2A; Supplemental Fig. S4). *EP300* was the predominant fusion partner ( $n = 6$ ), with its N terminus domains (amino acid residues 1 to 510) juxtaposed with the full-length *ZNF384* protein (Fig. 2A). As a result, the *EP300-ZNF384* fusion protein lacked the critical bromodomain and the HAT domain. A similar fusion was also observed between *ZNF384* and *CREBBP* ( $n = 1$ ) (Fig. 2A), another member of the p300/CBP coactivator family. The *CREBBP-ZNF384* fusion lost most of the *CREBBP* domains related to HAT activity but retained the KIX domain that is responsible for CREB and MYB interaction (Dai et al. 1996; Radhakrishnan et al. 1997). Other *ZNF384* rearrangements involved *TCF3* and *TAF15* ( $n = 3$  and 1, respectively) (Fig. 2A). We next performed whole-exome sequencing of *ZNF384*-rearranged cases ( $n = 10$ ) with available tumor, and matched germline samples to identify additional genomic abnormalities. An average of 4.3 nonsilent point mutations or insertions/deletions were observed per case (range from one to 11) (Fig. 2B; Supplemental Table S3), with no significant differences in frequency by *ZNF384* fusion partners. Somatic mutations in epigenetic genes were common in this group of ALL, e.g., *CREBBP* ( $n = 2$ ), *KDM6A* ( $n = 2$ ), *CHD4* ( $n = 1$ ), and *CHD8* ( $n = 1$ ). In particular, *CREBBP* mutations were only observed in cases with *TCF3*- and *TAF15-ZNF384* fusions, mutually exclusive with *EP300* or *CREBBP* translocations (Fig. 2B). Taken together, genomic lesions (mutation or translocation) of epigenetic regulator genes were present in 81.8% of *ZNF384*-rearranged ALL. Mutations in *RAS-PI3K* pathway were also noted, e.g., *PIK3CD*, *PTPN11*, *RIT1*, and *FLT3* (Supplemental Table S3). In an independent validation cohort of 602 Chinese children with newly diagnosed ALL (including 239



**Figure 1.** Whole-transcriptome sequencing of childhood ALL. (A) Unsupervised hierarchical clustering of global gene expression profile from 231 children with newly diagnosed ALL. Columns indicate ALL patients, and rows are genes; gene overexpression and underexpression are shown in red and green, respectively. ALL immunophenotype, cytogenetics, and selected chromosomal translocations are indicated for each sample *above* the heatmap, and the text *below* denotes seven major ALL subgroups identified on the basis of gene expression profiles. “Predominant fusion” refers to likely functional and driver fusion event, as defined in the Methods and Supplemental Figure S3A. (B) Pie chart of major gene fusions and cytogenetic abnormalities identified in B-ALL and T-ALL. Percentage indicates prevalence in the entire cohort; B-ALL and T-ALL are discriminated by solid fill and dot pattern, respectively. (C) Circos plot of the predominant fusions involving frequently translocated genes (i.e., genes with three or more different translocation partners identified). The line links the two partner genes in a fusion, with line width indicative of the prevalence of the specific fusion.

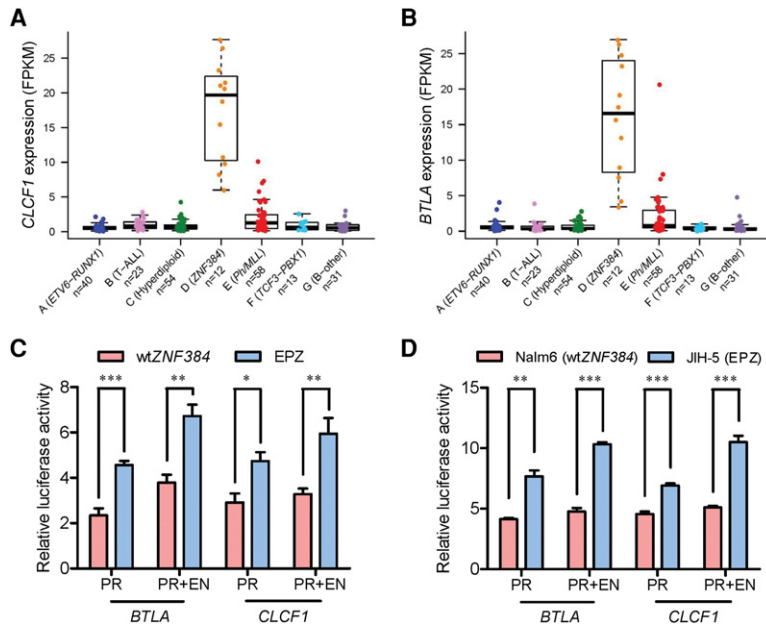
cases with *BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1*, *TCF3-HLF*, or *MLL* [also called *KMT2A*] rearrangements), we observed comparable frequencies of *EP300-ZNF384* (2.0%,  $n = 12$ ) and *CREBBP-ZNF384* fusions (1.8%,  $n = 11$ ) as detected by RT-PCR and Sanger sequencing. Our subsequent functional studies primarily focused on *EP300-ZNF384* and *CREBBP-ZNF384* fusions because of their predominance in *ZNF384*-rearranged ALL.

In spite of the variety of fusion partners, *ZNF384*-rearranged ALL was characterized by a common gene expression signature (Fig. 2C). Of 353 genes differentially expressed in this ALL subtype,

there was a significant enrichment of *ZNF384* binding sites as determined by ChIP-seq in B-lymphoblastoid cells (Rosenbloom et al. 2013), particularly in the promoter and/or enhancer regions ( $P = 6.8 \times 10^{-14}$ , Fisher’s exact test) (Supplemental Table S4). Two of the most significantly up-regulated genes in this group were *CLCF1* and *BTLA*, expressed at an average of 15.5- to 15.0-fold higher than cases with wild-type *ZNF384*, respectively (Fig. 3A, B). *CLCF1* encodes cardiotrophin-like-cytokine factor 1, which binds to CRLF1 to form a compound cytokine, eventually leading to activation of the JAK-STAT signaling and B-cell proliferation in







**Figure 3.** ZNF384 fusions transactivated CLCF1 and BTLA expression. (A,B) Expression of the CLCF1 (A) and BTLA (B) genes in seven ALL subgroups identified from hierarchical clustering. Each sample is represented by a dot and is color-coded according to the subgroups it belongs to. (C,D) Luciferase reporter gene assay of BTLA and CLCF1 promoter/enhancer activity. HEK293T cells were transiently transfected with pGL3 construct (luciferase gene with BTLA or CLCF1 promoter [PR] and/or enhancer [EN]), pcDNA construct (wild-type ZNF384 [wtZNF384] or EP300-ZNF384 [EPZ]), and pGL-TK (*Renilla* luciferase) (C). Similar sets of reporter gene assay were performed in the ALL cell lines Nalm6 (wtZNF384) and JIH-5 (EPZ) (D). Firefly luciferase activity was measured 24 h post-transfection and normalized to *Renilla* luciferase activity. Relative luciferase activity indicates the ratio over the value from pGL3 basic vector alone. All experiments were performed in triplicate and repeated at least three times. (PR) Promoter; (EN) enhancer; (wtZNF384) wild-type ZNF384; (EPZ) EP300-ZNF384 fusion. Statistical significance was evaluated by using two-sided Student's *t*-test: (\*)  $P < 0.05$ ; (\*\*)  $P < 0.01$ ; (\*\*\*)  $P < 0.001$ .

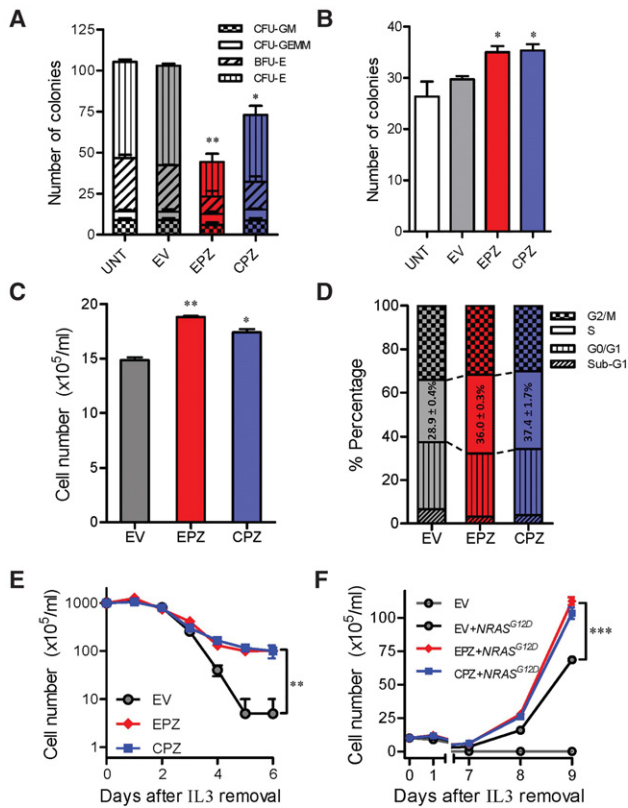
With IL3-independent growth as a measurement of oncogenic transformation, EP300- and CREBBP-ZNF384 fusions did not transform Ba/f3 cells in vitro, although with modest anti-apoptotic effects (Fig. 4E). We hypothesized that ZNF384 fusions are not oncogenic by themselves but instead may increase the transforming potential of other oncogenic mutations. In fact, Ba/f3 cells became IL3 independent 7 d upon cotransduction of ZNF384 fusion and NRAS<sup>G12D</sup>, significantly more quickly than cells expressing NRAS<sup>G12D</sup> alone (Fig. 4F). Similar effects were observed when EP300-ZNF384 or CREBBP-ZNF384 fusions were cotransduced with leukemogenic gene *CRLF2* (Supplemental Fig. S9).

Finally, we tested the effects of EP300-ZNF384 and CREBBP-ZNF384 fusions on histone modification. Wild-type (full-length), truncated, and fusion EP300 and CREBBP proteins were ectopically expressed in Sf9 insect cells, purified to homogeneity, and then subjected to HAT activity assay. EP300- and CREBBP-ZNF384 fusion proteins showed a 75% and 90% reduction of HAT activity of wild-type proteins, respectively, comparable to truncated EP300 and CREBBP proteins alone (Fig. 5A). This suggested that the loss of enzymatic activity was primarily due to the truncation of the HAT domain. The addition of fusion proteins (but not truncated EP300) also significantly repressed HAT activity of wild-type EP300 (Fig. 5B; Supplemental Fig. S10), indicating a dominant-negative effect that was dependent on fusion with ZNF384. Along the same line, expression of EP300- or CREBBP-ZNF384 fusion proteins significantly decreased global H3 acetylation (at both K9 and K27 residues) in Ba/f3 cells compared with the vector con-

trol (Fig. 5C; Supplemental Fig. S11). To test the feasibility of restoring histone acetylation as a therapeutic strategy for ALL cases with these ZNF384 fusions, we sought to evaluate the cytotoxicity of the histone deacetylase inhibitor vorinostat in Ba/f3 cells. NRAS<sup>G12D</sup>-transformed Ba/f3 cells underwent apoptosis upon vorinostat treatment in a dose-dependent fashion, but cotransduction of the EP300- or CREBBP-ZNF384 fusion significantly potentiated cytotoxic effects of histone deacetylase inhibition (Fig. 5D), with a concomitant increase in overall histone 3 acetylation (Supplemental Fig. S12). In parallel, in a panel of human ALL cell lines representative of common molecular subtypes (i.e., ETV6-RUNX1, TCF3-PBX1, BCR-ABL1, DUX4-IGH, MLL-rearranged, and ZNF384-rearranged), EP300-ZNF384-positive JIH-5 cells showed the lowest level of acetyl H3 at both the K9 and K27 residues and also the highest sensitivity to vorinostat compared with ALL cells with wild-type ZNF384 (Supplemental Fig. S13). Taken together, these results point to the possibility that ZNF384-rearranged ALL may benefit from therapeutic agents targeting histone acetylation regulation.

## Discussion

Chromosomal translocation in leukemia is arguably one of the first recognized genetic abnormalities in cancer (Rowley 2013). These nonrandom rearrangements are likely the founding events during leukemogenesis (Greaves 1997; Wiemels 2008). While focal genomic lesions (e.g., sequence mutations) can evolve during ALL progression and relapse (Mullighan et al. 2008; Yang et al. 2008; Ma et al. 2015), gene fusions arising from translocations usually remain unchanged across subclones over time, suggesting its fundamental role in the pathogenesis of this type of cancer (Anderson et al. 2011). By applying whole-transcriptome sequencing, we have identified a large number of fusion genes (Supplemental Table S2; Supplemental Fig. S3), and a few observations are particularly noteworthy. First, in this largely unselected cohort of childhood ALL, a readily expressed and predominant fusion gene was identified in 54.1% of patients, suggesting that gene fusion is a common form of genomic abnormality in ALL. Second, while most fusions were rare, a handful of genes (e.g., PAX5, ETV6, TCF3, ZNF384, and KMT2A) were repeatedly targeted by rearrangements with substantial diversity in their fusion partners; therefore, it is possible that a variety of mechanisms exist to deregulate these key genes during leukemogenesis. Third, of the 37 fusions (24 known and 13 novel) predicted to encode an in-frame chimeric protein and thus are most likely functional, many involved gene defects that may be therapeutically targeted (e.g., ABL1, CREBBP, PDGFR), highlighting opportunities for rationally designed novel ALL therapy. While we primarily focused on in-frame fusions, it is formally possible that "out-of-frame" fusions can lead to loss of function and are thus consequential in



**Figure 4.** *EP300*- and *CREBBP-ZNF384* fusions influence hematopoiesis and hematopoietic progenitor cell transformation in vitro. (A,B) Effects of fusion genes on mouse hematopoiesis in vitro. Mouse Lin<sup>-</sup>Sca1<sup>+</sup>c-Kit<sup>+</sup> cells were lentivirally transduced with empty vector (gray), *EP300-ZNF384* (red), or *CREBBP-ZNF384* (blue). Cells were then cultured in methylcellulose medium supplemented with cytokines for myelopoiesis (A) or pre-B-cell differentiation (B), and colony formation was assessed 12–14 d later. (C,D) *ZNF384* fusion genes and proliferation of mouse hematopoietic progenitor cell Ba/f3. Ba/f3 cells were lentivirally transduced with empty vector (grey), *EP300-ZNF384* (red), or *CREBBP-ZNF384* (blue) and then cultured in the presence of IL3 (10 ng/mL). After 48 h, cell density was examined using Trypan blue (C), and cell cycle distribution was evaluated using a standard PI staining protocol (D). (E,F) Effects of fusion genes on Ba/f3 transformation. Following transduction of empty vector or *ZNF384* fusion genes, Ba/f3 cells were cultured in IL3-depleted medium with cytokine-independent cell growth as a measure of oncogenic transformation. The number of viable cells was evaluated daily (E). A similar set of experiments was performed with cotransduction of oncogenic *NRAS*<sup>G12D</sup> (F). All experiments were performed in triplicate and repeated twice. (EV) Empty vector; (EPZ) *EP300-ZNF384* fusion; (CPZ) *CREBBP-ZNF384* fusion; (CFU-GM) colony-forming unit–granulocyte, macrophage; (CFU-GEMM) colony-forming unit–granulocyte, erythrocyte, macrophage, megakaryocyte; (BFU-E) burst-forming unit–erythroid; (CFU-E) colony-forming unit–erythroid. Statistical significance of the differences between EPZ versus EV or between CPZ versus EV was estimated by using two-sided Student's *t*-test (A–C) or two-way ANOVA (E,F): (\*)  $P < 0.05$ ; (\*\*)  $P < 0.01$ ; (\*\*\*)  $P < 0.001$ .

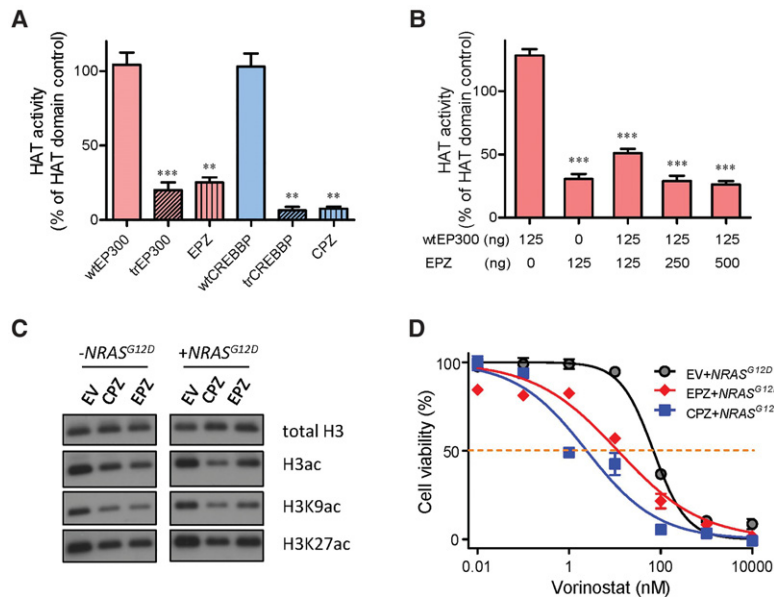
leukemogenesis. It should also be noted that our base-resolution whole-transcriptome sequencing enables a variety of genomic discoveries in addition to gene fusion detection and their associated gene expression signature. For example, future analyses of this data set would focus on novel transcript isoforms, allele-specific or -biased expression, etc. Finally, unlike most prior genomic profiling studies of ALL, our patient population is exclusively Asian and thus affords a unique opportunity to identify ancestry-related difference in the transcriptomic structure of ALL,

which should be considered in the future development of targeted therapy for this cancer.

The fact that *ZNF384* rearrangements are largely specific to ALL strongly argues that this gene plays a potentially important role in leukemogenesis even though little is known about the exact biological function of this zinc finger transcription factor. First identified for its direct interaction with focal adhesion protein BCAR1 (Janssen and Marynen 2006), *ZNF384* shuttles between cytoplasm and nucleus and can alter DNA structure in response to changes in cell shape and cytoskeleton architecture (Feister et al. 2000). Loss of *ZNF384* in vivo impairs bone formation (Morinobu et al. 2005) and leads to defects in spermatogenesis (Nakamoto et al. 2004), although the effects on hematopoiesis have not been comprehensively examined in these animal models. Across different hematopoietic compartments, *ZNF384* expression is fairly ubiquitous, with only modest down-regulation in hematopoietic stem cells (Novershtern et al. 2011), and also remains largely constant during B-cell differentiation (Hardy fractions A–F) (Supplemental Fig. S14) in contrast with other known transcription factors involved in hematopoiesis (Novershtern et al. 2011). *ZNF384*-rearranged ALLs exhibit immunophenotypes that are highly characteristic of Pro-B cells, suggesting that these fusion genes may specifically stall B-lymphocyte differentiation at this stage. Forced expression of *EP300-ZNF384* indeed inhibited mouse Pro-B cell differentiation in vivo (Yasuda et al. 2016), but in the more primitive HSPCs, this fusion protein blocked the differentiation along the myeloid and granulocyte lineages (Fig. 4A). Surprisingly, *ZNF384*-rearranged ALL often also expresses cell surface markers of myeloid lineage (CD13 and CD33) (Supplemental Table S1B), suggesting that *EP300-ZNF384* might function differently depending upon the exact precursor cell population in which it occurs. We postulate that the juxtaposition of the partner gene with *ZNF384* in the fusion protein confers novel functions that may interfere with normal hematopoiesis at multiple stages. So far, five partner genes (*EP300*, *CREBBP*, *TAF15*, *TCF3*, and *ESW1*) (Martini et al. 2002; Zhong et al. 2008; Nyquist et al. 2011; Gocho et al. 2015) have been identified in *ZNF384*-rearranged ALL, and it remains unclear whether there is a common mechanism for leukemogenesis in these cases. Our preliminary data thus far indicated that at least the *TAF15*- and *TCF3-ZNF384* fusions also positively regulated *BTLA* and *CLCF1* transcription and potentiated oncogenic transforming effects in Ba/f3 cells (especially with *CRLF2*), although with distinctive effects on HSPC function in vitro compared with *EP300*- and *CREBBP-ZNF384* fusions (Supplemental Figs. S7, S15). In fact, expression of *ZNF384* was also significantly higher in cases with *ZNF384* fusions than those without ( $P < 0.01$ , two-sided Student's *t*-test) (Supplemental Fig. S16). Taken together, these findings suggest the gain of *ZNF384* transcription factor activity as a common feature in *ZNF384* fusion functions but also point to differential effects possibly driven by the specific fusion partner genes involved. The contribution of *ZNF384* fusions to ALL leukemogenesis is likely to be multifactorial, and future mechanistic studies are warranted to determine the exact requirement of each functionality of these fusions.

While *ZNF384* rearrangement is predominantly observed in B-ALL, these leukemia blast cells also often express myeloid markers (La Starza et al. 2005; Gocho et al. 2015; Ping et al. 2015), pointing to its possible link to biphenotypic leukemia. Clinically, *ZNF384*-rearranged ALLs do not exhibit other unique presenting features and tend to be associated with a favorable prognosis in retrospective studies. In the context of the multi-institutional





**Figure 5.** *EP300*- and *CREBBP-ZNF384* fusions resulted in loss of HAT activity, global histone acetylation deregulation, and sensitivity to HDAC inhibition. (A,B) HAT enzymatic activity was measured for various *EP300* and *CREBBP* proteins: wild-type, truncated, and *ZNF384* fusions. Proteins were expressed in Sf9 insect cells and purified to homogeneity. (A) HAT activity was determined for each protein individually. (B) In parallel, wild-type *EP300* was mixed with increasing amount of *EP300-ZNF384* fusion protein to examine dominant-negative effects of the latter on HAT activity. (C) Global H3, H3K9, and H3K27 acetylation were evaluated by Western blot in Ba/f3 cells overexpressing *EP300-/CREBBP-ZNF384* fusion, with total H3 as a loading control. (D) Cytotoxicity of HDAC inhibitor vorinostat was examined in *NRAS<sup>G12D</sup>*-transformed Ba/f3 cells expressing *EP300-ZNF384* (red) or *CREBBP-ZNF384* (blue) or transduced with empty vector (gray). Cells were incubated with vorinostat for 48 h, and viability was then measured using a MTT assay. Experiments were performed in triplicate and repeated at least three times. (wtEP300) Wild-type *EP300*; (trEP300) truncated *EP300*; (wtCREBBP) wild-type *CREBBP*; (trCREBBP) truncated *CREBBP*; (EPZ) *EP300-ZNF384* fusion; (CPZ) *CREBBP-ZNF384* fusion; (EV) empty vector. EPZ- or CPZ-transduced cells were significantly more sensitive to vorinostat than EV, as determined by two-way ANOVA ( $P < 0.001$ ). (A,B) Statistical significance was evaluated by using a two-sided Student's *t*-test: (\*\*\*)  $P < 0.001$ ; (\*\*\*)  $P < 0.001$ .

prospective Ma-Spore frontline ALL trial, *ZNF384* fusions had no significant effects on prednisone treatment in vivo, leukemia burden at the end of induction therapy, or event-free survival (Supplemental Table S1A), although this could have been influenced by our relatively limited sample size.

*EP300* and *CREBBP* are two highly related proteins best known for their lysine acetyltransferase activity especially in the context of transcription coactivation (Ait-Si-Ali et al. 1998; Iyer et al. 2004; Liu et al. 2008). Canonically, *EP300* and *CREBBP* can directly influence chromatin conformation by targeted histone lysine acetylation (Liu et al. 2008), and concomitant *EP300* binding and H3K27 acetylation is a hallmark of promoter/enhancer activation (Vo and Goodman 2001). During hematopoiesis, *EP300* and *CREBBP* are involved in a complex series of transcriptional events mainly through interactions with other hematopoietic transcription factors (e.g., *GATA1*, *TCF3*, and *RUNX1*) (Blobel 2000). Leukemia-associated translocations involving *CREBBP* have been identified in acute myeloid leukemia (*KAT6A-CREBBP* and *KMT2A-CREBBP*) (Yang 2004), whereas *EP300* translocation is exceedingly rare, and in these cases, the fusion proteins retain most of the *EP300/CREBBP* functional domains. In contrast, in lymphoid malignancies, *CREBBP* was most frequently targeted by sequence mutation or focal copy number changes that mainly affect the HAT catalytic domain (Mullighan et al. 2011; Pasqualucci et al. 2011). Although uncommon in leukemia,

*EP300* mutations are also somewhat enriched in the HAT domain (Shigeno et al. 2004). In *ZNF384*-rearranged ALL, nearly all cases harbored genomic lesions in *EP300* and/or *CREBBP* that would lead to loss of HAT activity, suggesting that the selective truncation of this domain is critical. The global decrease of H3 acetylation that possibly resulted from dominant-negative *EP300-ZNF384* fusion also presented a potential therapeutic opportunity for small molecules such as histone deacetylase inhibitors (Fig. 5; Supplemental Fig. S13). Plausibly, histone deacetylation is required for leukemia maintenance in these ALL cases, thus restoring acetyl marks by vorinostat is anti-leukemic. While our data indicated a correlation between *EP300-ZNF384* fusion, aberrant histone acetylation, and sensitivity to vorinostat, future studies are needed to establish the causal relationship and delineate the exact mechanistic details. The function of *EP300/CREBBP* is incredibly complex, and therefore, deregulation of histone acetylation might be one of many mechanisms that link these fusions to leukemogenesis and sensitivity to this class of drugs.

In conclusion, we have comprehensively described gene fusions in childhood ALL and identified *ZNF384*-rearranged ALL as a distinct subtype with characteristic genomic lesions targeting epigenetic modulator genes. These findings shed lights on genomic

complexity of ALL and also potential genomics-guided therapeutic options.

## Methods

### Patients and samples

Included in this study were 231 children with newly diagnosed ALL treated in Ma-Spore frontline ALL clinical trials (Yeoh et al. 2012). Gross cytogenetics and common translocations were determined at the time of diagnosis (i.e., *BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1*, *STIL-TAL1*, *MLL* rearrangement, and hyperdiploid karyotype) by karyotyping and reverse transcription PCR in the reference laboratory at National University Hospital, Singapore (Yeoh et al. 2012). Subjects were selected based on sample availability. To test the frequency of *EP300-ZNF384* and *CREBBP-ZNF384* fusions, 602 children with newly diagnosed ALL from Beijing Children's Hospital, China (Gao et al. 2012), were included as the validation cohort (239 cases with *BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1*, *TCF3-HLF*, or *MLL* rearrangements). Total RNA and genomic DNA were extracted from diagnostic bone marrow. Peripheral blood or bone marrow specimens collected during clinical remission were used to prepare germline genomic DNA whenever applicable. This study was approved by the institutional review boards at St. Jude Children's Research Hospital, USA; National University of Singapore, Singapore; and Beijing Children's Hospital, China.

## Whole-transcriptome sequencing

TruSeq stranded mRNA library prep kit (Illumina) was used for whole-transcriptome library preparation, and paired-end sequencing was performed using the Illumina HiSeq 2000/2500 platform with a 101-bp read length at Hartwell Center of Biotechnology at St. Jude Children's Research Hospital. Sequencing reads were aligned to the human genome (hg19) reference sequence using TopHat2 (v2.0.12) (Trapnell et al. 2012). Samples were excluded if the percentage of exonic regions with 10× or more coverage was <30%, read duplication rate >55%, or a strong 3'-bias of transcript coverage. Relative transcript expression levels (fragments per kilobase of transcript per million mapped reads [FPKM]) were estimated based on supporting reads. For each gene, expression was summarized as the sum of FPKMs for all transcript isoforms and normalized across all qualified samples by Cuffnorm (v2.2.1, with the default geometric algorithm) (Trapnell et al. 2012) and by loess function of R stats package (version 3.1.3) (R Core Team 2015). The FPKM values were log<sub>2</sub> transformed for all subsequent analyses. Unsupervised hierarchical clustering (Ward.D2 method for clustering with Euclidean distance for samples and correlation for genes) (Murtagh and Legendre 2014) was performed after excluding genes with invariable expression (coefficient of variation <1) or extremely low or high expression (mean log<sub>2</sub>[FPKM + 1] < 0.585 or maximal log<sub>2</sub>[FPKM + 1] > 8, respectively). As a result, 490 most variably expressed genes were included in the final clustering and principal component analyses. Genes differentially expressed by ALL subgroup were identified using the SAM algorithm with fold change of more than two and false-discovery rate (*q*-value) <0.1% (Tusher et al. 2001). By using ZNF384 ChIP-seq data in the lymphoblastoid GM12878 cell line from ENCODE Project (Rosenbloom et al. 2013), we identified putative ZNF384 binding sites in genes differentially expressed in the ZNF384-rearranged ALL following previously established procedures (Qian et al. 2013).

Gene fusion was identified by two independent algorithms, namely, TopHat-Fusion (Kim and Salzberg 2011) and CICERO (Roberts et al. 2014). The initial screen for fusion transcript was performed using TopHat-Fusion with default parameters and predicted fusion genes involving mitochondria chromosome or with breakpoint-spanning read count of less than three in all suspected samples were excluded. In parallel, sequence reads were realigned by using the STAR (v2.4.0d) (Dobin et al. 2013) program and then analyzed by using the CICERO algorithm. Fusions called by CICERO with a high-confidence score (more than 400) were also added to candidate fusions identified by TopHat-Fusion. Manual review was applied to generate the final fusion gene list.

*ETV6-RUNX1*, *TCF3-PBX1*, *BCR-ABL1*, *STIL-TAL1*, and *MLL* fusions were screened during ALL diagnostic work-up, and we observed 100% concordance with the whole-transcriptome sequencing results. Fusions involving *ZNF384*, *ETV6*, *PAX5*, or *RUNX1* were prioritized for validation by PCR amplification of breakpoint region of the chimeric transcript in the corresponding patient cDNA, followed by Sanger sequencing for final confirmation (PCR and sequencing primers are listed in Supplemental Table S5). The remainders of the fusions were validated by PCR on the basis of gene function (i.e., *SPI1*, *MYB*, *LMO1*, *RAG1*, and *ABL1*) and sample availability. In total, 74 fusions in 125 samples were validated. We subjectively classified fusions into "predominant" versus "minor" to indicate the likelihood of a functional event versus a random and passenger event, following a multistep process considering predicted open reading frame of the chimeric transcript, adequate expression of the fusion, and also involvement of gene paralogs (Supplemental Fig. S3A). Wherever applicable, chromosomal translocations were visualized using Circos (Krzywinski et al. 2009).

## Whole-exome sequencing

Matched germline and tumor DNA from ALL cases with *ZNF384* rearrangements were subjected to whole-exome sequencing. The Nextera rapid capture expanded exome kit was used for capture and library preparation with insert size ~200 bp predominantly (ranges from 100–800 bp). Paired-end sequencing (2 × 101 bp read length) was performed using the Illumina HiSeq 2000/2500 platform at St. Jude Children's Research Hospital. Reads were aligned to the human genome (GRCh37) reference sequence (human\_g1k\_v37.fasta) using Burrows-Wheeler aligner MEM algorithm (version 0.7.12) (Li and Durbin 2009). Across 10 tumor-germline pairs (20 samples), we observed a median of 88% of exonic regions covered by at least 10× and 69% exome by at least 20×. All steps of the data processing prior to variant calling were performed according to the GATK best practices using GATK (version 3.5) (McKenna et al. 2010; DePristo et al. 2011) and Picard tools (version 1.141; <http://broadinstitute.github.io/picard>). Somatic mutations (single-nucleotide variants and short insertion/deletions) were called by using MuTect2 (Cibulskis et al. 2013). Mutations with one of the following features in the tumor data were excluded: (1) The read depth was less than 20; (2) the mutant allele frequency was <20%; (3) all reads supporting the mutation call came from the same mapping direction; (4) two and more mutations called in the same sample clustered together (within a 30-bp window). A total of 56 mutations remained, all of which were reviewed manually using the Integrative Genomics Viewer (Robinson et al. 2011). Twenty-one mutations were deemed false positive because of read mapping error due to genomic sequence duplication and thus removed from subsequent analyses. To address the possibility of variants missed by whole-exome sequencing due to low coverage in some cases, we also curated variant calls from the matched whole-transcriptome sequencing data. In fact, eight mutations originally excluded because of low mutant allele frequency or low read depth in whole-exome sequencing were validated in whole-transcriptome sequencing and thus confirmed as somatic mutation. The final list thus consisted of 43 high-confidence somatic mutations.

## ZNF384 target gene promoter and enhancer activity by luciferase assays

Full-length chimeric transcripts (*EP300*-, *CREBBP*-, *TCF3*-, and *TAF15-ZNF384*) were amplified from patient cDNA and cloned into pcDNA3.1+/Hydro(+) vector (Thermo Fisher Scientific) with a C terminus Flag tag. Putative promoter and enhancers of *BTLA* and *CLCF1* were identified from ENCODE ChIP-seq data in GM12878 cell line and were cloned into pGL3 luciferase vector (Promega): *BTLA* promoter, Chr 3: 112217785–112218842; *BTLA* enhancer, Chr 3: 112208658–112209560; *CLCF1* promoter, Chr 11: 67140939–67141906; and *CLCF1* enhancer, Chr 11: 67135923–67136631. To ensure specificity of these assays, we also tested pGL3 constructs void of predicted ZNF384-binding sites at the *BTLA* and *CLCF1* loci (deletion of sequence Chr 3: 112218257–112218467 within *BTLA* promoter and Chr 3: 112208991–112209202 within *BTLA* enhancer; deletion of sequence Chr 11: 67141234–67141499 within *CLCF1* promoter and Chr 11: 67136225–67136404 within *CLCF1* enhancer). All chromosomal coordinates were according to human genome hg19.

For the luciferase assay,  $2.5 \times 10^4$  HEK293T cells cultured in a 96-well plate were transiently transfected with 50 ng of pGL3 construct (luciferase gene with promoter and/or enhancer of interest), 50 ng of pcDNA construct (wild-type *ZNF384* or *ZNF384* fusion), and 5 ng of pGL-TK (*Renilla* luciferase) using Lipofectamine 2000 (Invitrogen). Similar luciferase assays were performed in leukemia



cell lines Nalm6 and JIH-5. Nalm6 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ), and the JIH-5 cells (Ping et al. 2015) were a gift from Dr. Suning Chen at Soochow Children's Hospital and Dr. Hans G. Drexler at DSMZ. Nalm6 or JIH-5 cells ( $1 \times 10^6$ ) were transiently transfected using Amaxa cell line nucleofector kit T (Lonza). Firefly luciferase activity was measured after 24 h post-transfection using the dual luciferase assay (Promega). The results were normalized to *Renilla* luciferase activity. These experiments were repeated at least three times, and each sample was assayed in triplicate.

### In vitro differentiation assay of mouse HSPCs

*EP300*-, *CREBBP*-, *TCF3*-, and *TAF15-ZNF384* transcripts were cloned into the pcL20c-IRES-GFP lentiviral vector, and lentiviral supernatants were produced by transient transfection of HEK293T cells using calcium phosphate (Xu et al. 2015). Lin<sup>-</sup>Sca1<sup>+</sup>c-Kit<sup>+</sup> HSPCs were sorted from bone marrow of C57/BL6 mice (8–10 wk old) by using flow cytometry (Taggart et al. 2016) and lentivirally transduced with *ZNF384* fusions for 72 h followed by GFP selection (Ye et al. 2015). For in vitro colony formation assays, 500 GFP<sup>+</sup> HSPCs were plated in 35-mm Petri dishes in Methocult M3434 or M3630 methylcellulose medium (Stemcell Technologies) to examine differentiation potential for myelopoiesis or into pre-B cells, respectively. Methocult M3434 medium consists of 2% methylcellulose, SCF (50 ng/mL), IL3 (10 ng/mL), IL6 (10 ng/mL), and EPO (3 U/mL), and M3630 is supplemented with IL7 (10 ng/mL). Serial replating was done at 500 cells per 35-mm Petri dish in the same medium above. The number of colonies was counted 12–14 d after plating.

### Mouse hematopoietic progenitor cell Ba/f3 proliferation and transformation assay

Flag-tagged *ZNF384* fusions were introduced into Ba/f3 cells by lentiviral transduction. GFP-positive cells were sorted 48 h later and plated at  $1 \times 10^5$  cells/mL density in the presence of 10 ng/mL IL3. Cell growth was monitored daily using Trypan blue, and cell cycle distribution was determined 48 h after plating using a standard propidium iodide (PI) staining protocol.

To examine transformation potential, Ba/f3 cells expressing different *ZNF384* fusion genes were further transduced with *NRAS*<sup>G12D</sup> (retroviral, pMSCV) or *CRLF2* (lentiviral, pcL20C). IL3 was removed 48 h later, and the number of viable cells was monitored daily by Trypan blue. The *NRAS*<sup>G12D</sup> and *CRLF2* constructs were gifts from Dr. Charles Mullighan at St. Jude Children's Research Hospital. Retroviral particles were produced using ecotropic HEK293T cells (American Type Culture Collection) (Xu et al. 2015). Each experiment was performed three times.

### HAT activity assay and global histone acetylation

Wild-type (full-length) and truncated *EP300* and *CREBBP*, *EP300-ZNF384* fusion, and *CREBBP-ZNF384* fusion were cloned into the pFastBac/HBM-TOPO vector with a C terminus His-tag. Each protein was ectopically expressed in Sf9 cells and purified to homogeneity by affinity chromatography (Invitrogen) (Supplemental Fig. S17; Delvecchio et al. 2013). His-tag was removed by AcTEV protease (Invitrogen) for all recombinant proteins, and HAT activity was then measured using HAT assay kit (Active Motif). The recombinant wild-type *EP300* HAT domain (Active Motif) was included as a positive control.

In transformed Ba/f3 cells, *ZNF384* fusion gene expression and histone H3 acetylation were evaluated by Western blot, using anti-acetyl-histone H3 antibody (Millipore, 06-599, 1:1000 dilution), anti-histone H3 antibody (Cell Signaling, 4499S, 1:1,000

dilution), anti-histone H3 (acetyl K9) antibody (Abcam, ab4441, 1:1,000 dilution), and anti-histone H3 (acetyl K27) antibody (Abcam, ab4729, 1:1,000 dilution). Histone H3 was used as a loading control. The relative quantification of immunoblots were analyzed using Image Studio lite version 4.0.21 (LI-COR Biosciences). Following the same procedures, six human ALL cell lines (JIH-5, Nalm6, SEM, 697, SUP-B15, and REH) representative of the major molecular subtypes were also assayed for histone acetylation by Western blotting. ALL cell lines were obtained from the American Type Culture Collection or DSMZ unless otherwise indicated.

### Vorinostat cytotoxicity

*NRAS*<sup>G12D</sup>-transformed Ba/f3 cells overexpressing *EP300-ZNF384* or *CREBBP-ZNF384* and the six human ALL cells described above were incubated with increasing concentrations of vorinostat for 48 h, after which cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Experiments were performed in triplicate and repeated at least three times, and results were plotted using Graph Prism 6.

### Statistical analysis

All statistical analyses were performed using Graphpad Prism and/or R (R Core Team 2015); all tests were two-sided, and the level of statistical significance was  $P < 0.05$ .

### Data access

Sequencing data have been submitted to the European Genome-Phenome Archive (EGA; <http://www.ebi.ac.uk/ega>), which is hosted by the European Bioinformatics Institute (EBI), under accession number EGAS00001001858.

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## References

- Abraham BJ, Cui K, Tang Q, Zhao K. 2013. Dynamic regulation of epigenomic landscapes during hematopoiesis. *BMC Genomics* **14**: 193.
- Ait-Si-Ali S, Ramirez S, Barre FX, Dkhissi F, Magnaghi-Jaulin L, Girault JA, Robin P, Knibiehler M, Pritchard LL, Ducommun B, et al. 1998. Histone acetyltransferase activity of CBP is controlled by cycle-dependent kinases and oncoprotein E1A. *Nature* **396**: 184–186.
- Anderson K, Lutz C, van Delft FW, Bateman CM, Guo Y, Colman SM, Kempinski H, Moorman AV, Tittley I, Swansbury J, et al. 2011. Genetic variegation of clonal architecture and propagating cells in leukaemia. *Nature* **469**: 356–361.
- Atak ZK, Gianfelici V, Hulselmans G, De Keersmaecker K, Devasia AG, Geerdens E, Mentens N, Chiaretti S, Durinck K, Uyttebroeck A, et al. 2013. Comprehensive analysis of transcriptome variation uncovers known and novel driver events in T-cell acute lymphoblastic leukemia. *PLoS Genet* **9**: e1003997.
- Balasubramani A, Larjo A, Bassein JA, Chang X, Hastie RB, Togher SM, Lahdesmaki H, Rao A. 2015. Cancer-associated *ASXL1* mutations may act as gain-of-function mutations of the ASXL1–BAP1 complex. *Nature Commun* **6**: 7307.
- Bateman CM, Alpar D, Ford AM, Colman SM, Wren D, Morgan M, Kearney L, Greaves M. 2015. Evolutionary trajectories of hyperdiploid ALL in monozygotic twins. *Leukemia* **29**: 58–65.
- Blobel GA. 2000. CREB-binding protein and p300: molecular integrators of hematopoietic transcription. *Blood* **95**: 745–755.
- Chalmers JJ, Kim E, Telford JN, Wong EY, Tacon WC, Shuler ML, Wilson DB. 1990. Effects of temperature on *Escherichia coli* overproducing  $\beta$ -lactamase or human epidermal growth factor. *Appl Environ Microbiol* **56**: 104–111.
- Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, Gabriel S, Meyerson M, Lander ES, Getz G. 2013. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol* **31**: 213–219.
- Crabe S, Guay-Giroux A, Tormo AJ, Duluc D, Lissilaa R, Guilhot F, Mavoungou-Bigouagou U, Lefouili F, Cognet I, Ferlin W, et al. 2009. The IL-27 p28 subunit binds cytokine-like factor 1 to form a cytokine regulating NK and T cell activities requiring IL-6R for signaling. *J Immunol* **183**: 7692–7702.
- Dai P, Akimaru H, Tanaka Y, Hou DX, Yasukawa T, Kanei-Ishii C, Takahashi T, Ishii S. 1996. CBP as a transcriptional coactivator of c-Myb. *Genes Dev* **10**: 528–540.
- Delvecchio M, Gaucher J, Aguilar-Gurrieri C, Ortega E, Panne D. 2013. Structure of the p300 catalytic core and implications for chromatin targeting and HAT regulation. *Nat Struct Mol Biol* **20**: 1040–1046.
- DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, et al. 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* **43**: 491–498.
- Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**: 15–21.
- Feister HA, Torungruang K, Thunyakitpisal P, Parker GE, Rhodes SJ, Bidwell JP. 2000. NP/NMP4 transcription factors have distinct osteoblast nuclear matrix subdomains. *J Cell Biochem* **79**: 506–517.
- Gao C, Zhao XX, Li WJ, Cui L, Zhao W, Liu SG, Yue ZX, Jiao Y, Wu MY, Li ZG. 2012. Clinical features, early treatment responses, and outcomes of pediatric acute lymphoblastic leukemia in China with or without specific fusion transcripts: a single institutional study of 1,004 patients. *Am J Hematol* **87**: 1022–1027.
- Gocho Y, Kiyokawa N, Ichikawa H, Nakabayashi K, Osumi T, Ishibashi T, Ueno H, Terada K, Oboki K, Sakamoto H, et al. 2015. A novel recurrent EP300–ZNF384 gene fusion in B-cell precursor acute lymphoblastic leukemia. *Leukemia* **29**: 2445–2448.
- Greaves MF. 1997. Aetiology of acute leukaemia. *Lancet* **349**: 344–349.
- Greaves MF, Wiemels J. 2003. Origins of chromosome translocations in childhood leukaemia. *Nat Rev Cancer* **3**: 639–649.
- Hong D, Gupta R, Ancliff P, Atzberger A, Brown J, Soneji S, Green J, Colman S, Piacibello W, Buckle V, et al. 2008. Initiating and cancer-propagating cells in *TEL-AML1*-associated childhood leukemia. *Science* **319**: 336–339.
- Huether R, Dong L, Chen X, Wu G, Parker M, Wei L, Ma J, Edmonson MN, Hedlund EK, Rusch MC, et al. 2014. The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. *Nat Commun* **5**: 3630.
- Hunger SP, Mullighan CG. 2015. Acute lymphoblastic leukemia in children. *N Engl J Med* **373**: 1541–1552.
- Iacobucci I, Li Y, Roberts KG, Dobson SM, Kim JC, Payne-Turner D, Harvey RC, Valentine M, McCastlain K, Easton J, et al. 2016. Truncating erythropoietin receptor rearrangements in acute lymphoblastic leukemia. *Cancer Cell* **29**: 186–200.
- Inaba H, Greaves M, Mullighan CG. 2013. Acute lymphoblastic leukaemia. *Lancet* **381**: 1943–1955.
- Iyer NG, Ozdag H, Caldas C. 2004. p300/CBP and cancer. *Oncogene* **23**: 4225–4231.
- Janssen H, Maryn P. 2006. Interaction partners for human ZNF384/CIZ/NMP4—zyxin as a mediator for p130CAS signaling? *Exp Cell Res* **312**: 1194–1204.
- Ji H, Ehrlich LI, Seita J, Murakami P, Doi A, Lindau P, Lee H, Aryee MJ, Irizarry RA, Kim K, et al. 2010. Comprehensive methylome map of lineage commitment from haematopoietic progenitors. *Nature* **467**: 338–342.
- Kannan S, Kurupati RK, Doyle SA, Freeman GJ, Schmader KE, Ertl HC. 2015. BTLA expression declines on B cells of the aged and is associated with low responsiveness to the trivalent influenza vaccine. *Oncotarget* **6**: 19445–19455.
- Kim D, Salzberg SL. 2011. TopHat-Fusion: an algorithm for discovery of novel fusion transcripts. *Genome Biol* **12**: R72.
- Kimbrel EA, Lemieux ME, Xia X, Davis TN, Rebel VI, Kung AL. 2009. Systematic in vivo structure-function analysis of p300 in hematopoiesis. *Blood* **114**: 4804–4812.
- Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. 2009. Circos: an information aesthetic for comparative genomics. *Genome Res* **19**: 1639–1645.
- La Starza R, Aventin A, Crescenzi B, Gorello P, Specchia G, Cuneo A, Angioni A, Billoh-Nabera C, Boque C, Foa R, et al. 2005. *CIZ* gene rearrangements in acute leukemia: report of a diagnostic FISH assay and clinical features of nine patients. *Leukemia* **19**: 1696–1699.
- Lara-Astiaso D, Weiner A, Lorenzo-Vivas E, Zaretzky I, Jaitin DA, David E, Keren-Shaul H, Mildner A, Winter D, Jung S, et al. 2014. Immunogenetics. Chromatin state dynamics during blood formation. *Science* **345**: 943–949.
- Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**: 1754–1760.
- Liu X, Wang L, Zhao K, Thompson PR, Hwang Y, Marmorstein R, Cole PA. 2008. The structural basis of protein acetylation by the p300/CBP transcriptional coactivator. *Nature* **451**: 846–850.
- Llinas L, Lazaro A, de Salort J, Matesanz-Isabel J, Sintes J, Engel P. 2011. Expression profiles of novel cell surface molecules on B-cell subsets and plasma cells as analyzed by flow cytometry. *Immunol Lett* **134**: 113–121.
- Ma X, Edmonson M, Yergeau D, Muzny DM, Hampton OA, Rusch M, Song G, Easton J, Harvey RC, Wheeler DA, et al. 2015. Rise and fall of subclones from diagnosis to relapse in pediatric B-acute lymphoblastic leukaemia. *Nat Commun* **6**: 6604.
- Mar BG, Bullinger LB, McLean KM, Grauman PV, Harris MH, Stevenson K, Neuberg DS, Sinha AU, Sallan SE, Silverman LB, et al. 2014. Mutations in epigenetic regulators including SETD2 are gained during relapse in paediatric acute lymphoblastic leukaemia. *Nat Commun* **5**: 3469.
- Martini A, La Starza R, Janssen H, Billoh-Nabera C, Corveleyn A, Somers R, Aventin A, Foa R, Hagemeyer A, Mecucci C, et al. 2002. Recurrent rearrangement of the Ewing's sarcoma gene, *EWSR1*, or its homologue, *TAF15*, with the transcription factor *CIZ/NMP4* in acute leukemia. *Cancer Res* **62**: 5408–5412.
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytzky A, Garimella K, Altshuler D, Gabriel S, Daly M, et al. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* **20**: 1297–1303.
- Morinobu M, Nakamoto T, Hino K, Tsuji K, Shen ZJ, Nakashima K, Nifuji A, Yamamoto H, Hirai H, Noda M. 2005. The nucleocytoplasmic shuttling protein CIZ reduces adult bone mass by inhibiting bone morphogenetic protein-induced bone formation. *J Exp Med* **201**: 961–970.
- Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, Girtman K, Mathew S, Ma J, Pounds SB, et al. 2007. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* **446**: 758–764.
- Mullighan CG, Phillips LA, Su X, Ma J, Miller CB, Shurtleff SA, Downing JR. 2008. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. *Science* **322**: 1377–1380.
- Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, Heatley SL, Holmfeldt L, Collins-Underwood JR, Ma J, et al. 2011. *CREBBP* mutations in relapsed acute lymphoblastic leukaemia. *Nature* **471**: 235–239.
- Murtagg F, Legendre P. 2014. Ward's hierarchical agglomerative clustering method: Which algorithms implement Ward's criterion? *J Classif* **31**: 274–295.
- Nakamoto T, Shiratsuchi A, Oda H, Inoue K, Matsumura T, Ichikawa M, Saito T, Seo S, Maki K, Asai T, et al. 2004. Impaired spermatogenesis and male fertility defects in *CIZ/Nmp4*-disrupted mice. *Genes Cells* **9**: 575–589.
- Nikolovski G, Langemeijer SM, Kuiper RP, Knops R, Massop M, Tonissen ER, van der Heijden A, Scheele TN, Vandenbergh P, de Witte T, et al. 2010.

- Somatic mutations of the histone methyltransferase gene *EZH2* in myelodysplastic syndromes. *Nat Genet* **42**: 665–667.
- Novershtern N, Subramanian A, Lawton LN, Mak RH, Haining WN, McConkey ME, Habib N, Yosef N, Chang CY, Shay T, et al. 2011. Densely interconnected transcriptional circuits control cell states in human hematopoiesis. *Cell* **144**: 296–309.
- Nyquist KB, Thorsen J, Zeller B, Haaland A, Troen G, Heim S, Micci F. 2011. Identification of the *TAF15-ZNF384* fusion gene in two new cases of acute lymphoblastic leukemia with a t(12;17)(p13;q12). *Cancer Genet* **204**: 147–152.
- Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, Kasper LH, Lerach S, Tang H, Ma J, et al. 2011. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature* **471**: 189–195.
- Ping N, Qiu H, Wang Q, Dai H, Ruan C, Ehrentraut S, Drexler HG, MacLeod RA, Chen S. 2015. Establishment and genetic characterization of a novel mixed-phenotype acute leukemia cell line with EP300-ZNF384 fusion. *J Hematol Oncol* **8**: 100.
- Pui CH, Yang JJ, Hunger SP, Pieters R, Schrappe M, Biondi A, Vora A, Baruchel A, Silverman LB, Schmiegelow K, et al. 2015. Childhood acute lymphoblastic leukemia: progress through collaboration. *J Clin Oncol* **33**: 2938–2948.
- Qian M, Jin W, Zhu X, Xia X, Yang X, Du Y, Wang K, Zhang J. 2013. Structurally differentiated *cis*-elements that interact with PU.1 are functionally distinguishable in acute promyelocytic leukemia. *J Hematol Oncol* **6**: 25.
- R Core Team. 2015. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Radhakrishnan I, Perez-Alvarado GC, Parker D, Dyson HJ, Montminy MR, Wright PE. 1997. Solution structure of the KIX domain of CBP bound to the transactivation domain of CREB: a model for activator:coactivator interactions. *Cell* **91**: 741–752.
- Roberts KG, Mullighan CG. 2015. Genomics in acute lymphoblastic leukaemia: insights and treatment implications. *Nat Rev Clin Oncol* **12**: 344–357.
- Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang YL, Pei D, McCastlain K, Ding L, Lu C, Song G, et al. 2014. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med* **371**: 1005–1015.
- Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. 2011. Integrative genomics viewer. *Nat Biotechnol* **29**: 24–26.
- Robison LL. 2011. Late effects of acute lymphoblastic leukemia therapy in patients diagnosed at 0–20 years of age. *Hematology* **2011**: 238–242.
- Rosenbloom KR, Sloan CA, Malladi VS, Dreszer TR, Learned K, Kirkup VM, Wong MC, Maddren M, Fang R, Heitner SG, et al. 2013. ENCODE data in the UCSC Genome Browser: year 5 update. *Nucleic Acids Res* **41**: D56–D63.
- Rowley JD. 2013. Genetics. A story of swapped ends. *Science* **340**: 1412–1413.
- Savin VJ, Sharma M, Zhou J, Gennochi D, Fields T, Sharma R, McCarthy ET, Srivastava T, Domen J, Tormo A, et al. 2015. Renal and hematological effects of CLCF-1, a B-cell-stimulating cytokine of the IL-6 family. *J Immunol Res* **2015**: 714964.
- Schindler JW, Van Buren D, Foudi A, Krejci O, Qin J, Orkin SH, Hock H. 2009. TEL-AML1 corrupts hematopoietic stem cells to persist in the bone marrow and initiate leukemia. *Cell Stem Cell* **5**: 43–53.
- Senaldi G, Stolina M, Guo J, Faggioni R, McCabe S, Kaufman SA, Van G, Xu W, Fletcher FA, Boone T, et al. 2002. Regulatory effects of novel neurotrophin-1/B cell-stimulating factor-3 (cardiotrophin-like cytokine) on B cell function. *J Immunol* **168**: 5690–5698.
- Shigeno K, Yoshida H, Pan L, Luo JM, Fujisawa S, Naito K, Nakamura S, Shinjo K, Takeshita A, Ohno R, et al. 2004. Disease-related potential of mutations in transcriptional cofactors CREB-binding protein and p300 in leukemias. *Cancer Lett* **213**: 11–20.
- Sims NA. 2015. Cardiotrophin-like cytokine factor 1 (CLCF1) and neurotrophin (NP) signalling and their roles in development, adulthood, cancer and degenerative disorders. *Cytokine Growth Factor Rev* **26**: 517–522.
- Stergachis AB, Neph S, Reynolds A, Humbert R, Miller B, Paige SL, Vernot B, Cheng JB, Thurman RE, Sandstrom R, et al. 2013. Developmental fate and cellular maturity encoded in human regulatory DNA landscapes. *Cell* **154**: 888–903.
- Taggart J, Ho TC, Amin E, Xu H, Barlowe TS, Perez AR, Durham BH, Tivnan P, Okabe R, Chow A, et al. 2016. MS12 is required for maintaining activated myelodysplastic syndrome stem cells. *Nat Commun* **7**: 10739.
- Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L. 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* **7**: 562–578.
- Tsuzuki S, Seto M. 2013. TEL (ETV6)-AML1 (RUNX1) initiates self-renewing fetal pro-B cells in association with a transcriptional program shared with embryonic stem cells in mice. *Stem Cells* **31**: 236–247.
- Tusher VG, Tibshirani R, Chu G. 2001. Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci* **98**: 5116–5121.
- Vendel AC, Calemene-Fenaux J, Izrael-Tomasevic A, Chauhan V, Arnott D, Eaton DL. 2009. B and T lymphocyte attenuator regulates B cell receptor signaling by targeting Syk and BLNK. *J Immunol* **182**: 1509–1517.
- Vo N, Goodman RH. 2001. CREB-binding protein and p300 in transcriptional regulation. *J Biol Chem* **276**: 13505–13508.
- Wiemels J. 2008. Chromosomal translocations in childhood leukemia: natural history, mechanisms, and epidemiology. *J Natl Cancer Inst Monogr* **2008**(39): 87–90.
- Xu H, Zhang H, Yang W, Yadav R, Morrison AC, Qian M, Devidas M, Liu Y, Perez-Andreu V, Zhao X, et al. 2015. Inherited coding variants at the *CDKN2A* locus influence susceptibility to acute lymphoblastic leukaemia in children. *Nat Commun* **6**: 7553.
- Yang XJ. 2004. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Res* **32**: 959–976.
- Yang JJ, Bhojwani D, Yang W, Cai X, Stocco G, Crews K, Wang J, Morrison D, Devidas M, Hunger SP, et al. 2008. Genome-wide copy number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukemia. *Blood* **112**: 4178–4183.
- Yasuda T, Tsuzuki S, Kawazu M, Hayakawa F, Kojima S, Ueno T, Imoto N, Kohsaka S, Kunita A, Doi K, et al. 2016. Recurrent *DUX4* fusions in B cell acute lymphoblastic leukemia of adolescents and young adults. *Nat Genet* **48**: 569–574.
- Ye M, Zhang H, Yang H, Koche R, Staber PB, Cusan M, Levantini E, Welner RS, Bach CS, Zhang J, et al. 2015. Hematopoietic differentiation is required for initiation of acute myeloid leukemia. *Cell Stem Cell* **17**: 611–623.
- Yeoh AE, Ariffin H, Chai EL, Kwok CS, Chan YH, Ponnudurai K, Campana D, Tan PL, Chan MY, Kham SK, et al. 2012. Minimal residual disease-guided treatment deintensification for children with acute lymphoblastic leukemia: results from the Malaysia-Singapore acute lymphoblastic leukemia 2003 study. *J Clin Oncol* **30**: 2384–2392.
- Zhong CH, Prima V, Liang X, Frye C, McGavran L, Meltesen L, Wei Q, Boomer T, Varella-Garcia M, Gump J, et al. 2008. *E2A-ZNF384* and *NOL1-E2A* fusion created by a cryptic t(12;19)(p13.3; p13.3) in acute leukemia. *Leukemia* **22**: 723–729.
- Zhu X, He F, Zeng H, Ling S, Chen A, Wang Y, Yan X, Wei W, Pang Y, Cheng H, et al. 2014. Identification of functional cooperative mutations of *SETD2* in human acute leukemia. *Nat Genet* **46**: 287–293.

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